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Research article

Electrochemical effect on bioleaching of arsenic and manganese from tungsten mine wastes using *Acidithiobacillus* spp.

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ABSTRACT

Mine wastes from tungsten mine which contain a high concentration of arsenic (As) may expose many environmental problems because As is very toxic. This study aimed to evaluate bioleaching efficiency of As and manganese (Mn) from tungsten mine wastes using the pure and mixed culture of *Acidithiobacillus ferrooxidans* and *A. thiooxidans*. The electrochemical effect of the electrode through externally applied voltage on bacterial growth and bioleaching efficiency was also clarified. The obtained results indicated that both the highest As extraction efficiency (96.7%) and the highest Mn extraction efficiency (100%) were obtained in the mixed culture. *A. ferrooxidans* played a more important role than *A. thiooxidans* in the extraction of As whereas *A. thiooxidans* was more significant than *A. ferrooxidans* in the extraction of Mn. Unexpectedly, the external voltage applied to the bioleaching did not enhance metal extraction rate but inhibited bacterial growth, resulting in a reverse effect on bioleaching efficiency. This could be due to the low electrical tolerance of bioleaching bacteria. However, this study asserted that As and Mn could be successfully removed from tungsten mine waste by the normal bioleaching using the mixed culture of *A. ferrooxidans* and *A. thiooxidans*.

1. Introduction

Mine wastes from mining industry are always the burdensome problems for both mining operator and environmental agency. These mine wastes contain high amount of toxic metals and metalloids such as As, Sb, Zn, Cu, Pb, Ni, and Cr which could easily migrate to surrounding environment under weathering and microbial activities and result in serious contamination of soils, rivers, and groundwater (Lee et al., 2011; Nguyen and Lee, 2015a; Schippers et al., 2000). Among above toxic metals, As was concentrated during the selective recovery of precious metals, resulting in a high concentration of As in mine wastes from mining activities of precious metal mines (Ahn et al., 2005; Lee et al., 2015; Lim et al., 2009). As being listed as one of the metalloid carcinogens, the toxicology of As was fully investigated in the literature (Choong et al., 2007; Mandal and Suzuki, 2002; WHO, 2011).

Due to the potential hazard coming from mine tailings, the Korean government has initiated reclamations of abandoned mines by establishing concrete retaining walls in order to limit the physical migration of mine tailings (Ko et al., 2013; Lee et al., 2015). However, this method could not cut off the concern of chemical migration of toxic metals to surrounding environments. In many attempts to remove toxic metals from mine wastes using economical and eco-friendly methods, bioleaching was the most potential method due to its low cost and low environmental impact (Asghari et al., 2013; Bosecker, 1997; Brierley and Brierley, 2013; Fonti et al., 2016; Lee et al., 2015). This technology has been widely investigated for toxic metals removal from soils (Diaz et al., 2015; Huang et al., 2015; Nareshkumar et al., 2008), sediments (Akinci and Guven, 2011; Chen and Lin, 2009a; Hoque and Philip, 2011), and mine tailings (Lee et al., 2015; Liu et al., 2008; Seh-Bardan et al., 2012). Bioleaching was widely applied for removal and recovery of Mn from mining waste residues (Ghosh et al., 2018, 2016; Ghosh and Das, 2017; Mohanty et al., 2017; Sanket et al., 2017). Especially, bioleaching was also effective with mine tailings highly contaminated with As (Lee et al., 2015; Park et al., 2014) and As-containing minerals (Zhang et al., 2015, 2007). In bioleaching of As, the use of *Acidithiobacillus* spp. such as *A. thiooxidans* and *A. ferrooxidans* represented

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many advantages because these bacteria are highly resistant to As (Zhang et al., 2007). *A. ferrooxidans* used both Fe(II) and sulfur compounds such as elemental sulfur and thiosulfate as energy sources whereas *A. thiooxidans* can only use sulfur compounds for their growth (Brierley and Brierley, 2013; Vera et al., 2013).

Even though bioleaching is an advantageous process for remediation of the environment contaminated with toxic metals when compared to other physical and chemical processes (Nguyen and Lee, 2015b), bioleaching was limited by slow reaction rate and long operation time (Chen and Lin, 2009b; Nguyen and Lee, 2014; Zhou et al., 2015). Many approaches attempted to enhance bioleaching efficiency through increasing reaction rate and reduce operation time. Some metal ions, such as Ag, Hg, Co, Bi, Ru and activated carbon could enhance bioleaching efficiency (Ahmadi et al., 2013; Muñoz et al., 2007; Nguyen and Lee, 2014; Yuehua et al., 2002; Zhang and Gu, 2007). Bioelectrochemical system is the recent approach in environmental remediation, in which the biological reduction and oxidation of pollutants were accelerated by the externally applied voltage (Luo et al., 2014; Nguyen et al., 2016a, 2016b; Xie et al., 2014). The enhancement of bacterial growth by the external voltage of a bioelectrochemical system treating toluene was also indicated previously (Friman et al., 2012).

This study aims to enhance bioleaching efficiency using the electrochemical interaction between the electrode, microorganism, and mine waste in a bioelectrochemical leaching system. The microbial oxidations of Fe(II) and sulfur compounds in bioleaching were expected to be accelerated at high anode potentials when the external voltage of 0.5–1.5 V was applied. The hypothesis for catalytic enhancement of external anode on bioleaching rate was sketched in Supplementary Fig. S1. Before this investigation, the bioleaching of tungsten mine waste was investigated in flasks with the pure and mixed culture of *A. ferrooxidans* with *A. thiooxidans* in order to select the best inoculum for extraction of both As and Mn. The microscopic morphology of mine waste before and after bioleaching was also characterized. The kinetics of As and Mn solubilization rate was evaluated.

2. Materials and methods

2.1. Mine waste characterization

Mine waste was collected from a closed mine in Korea. Various samples collected from different points of mine waste storage area were mixed well to enhance homogeneity. The target metal of this mine was Tungsten. The particle size of the mine waste was determined by sieving method. In order to define major elemental contents, the mine waste was subjected to Energy Dispersive X-ray fluorescence (EDXRF) analyzer (Applied Rigaku Technologies, Inc., Austin, USA). The mine waste was also introduced to X-ray diffractometer (XRD; Empyrean series 2, PANanalytical B.V., Almelo, the Netherlands) with a Cu cathode and the scanning 2θ position in the range from 10 to 100° for determination of mineralogical components. The obtained XRD data were matched with the data of standard compounds in the database using the Match! Program version 3.5 (Crystal Impact GbR, Bonn, Germany). Microscopic morphology of the mine waste was characterized by an analytical high-resolution scanning electron microscope (SEM; Hitachi SU-70, Hitachi High-Technologies, Japan) which is coupled with an energy-dispersive X-ray spectroscopy (EDAX Inc., Mahwah, NJ, USA). In addition, the mine waste sample was digested by aqua regia (HNO_3:HCl = 1:3, v/v) and the liquor was analyzed for their total metal contents (Fe, Mn, and As) using an inductively coupled plasma–atomic emission spectroscopy (ICP-AES; Activa, JY Horiba, France).

2.2. Microbial inocula and acclimation

The cultures of *Acidithiobacillus ferrooxidans* (KCTC 4516) and *Acidithiobacillus thiooxidans* (KCTC 4515) were bought from Korean Collection for Type Cultures and continuously subcultured to use throughout this experiment. The bacterial cultures were cultivated in 250 mL Erlenmeyer flasks with a working volume of 100 mL medium 271 (Growth mediums of *Acidithiobacillus*, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) which contains $2\ g\ L^{-1}$ of $(NH_4)_2SO_4$, $0.5\ g\ L^{-1}$ of K_2HPO_4, $0.5\ g\ L^{-1}$ of $MgSO_4 \cdot 7H_2O$, $0.1\ g\ L^{-1}$ of KCl, and $0.02\ g\ L^{-1}$ of $Ca(NO_3)_2 \cdot 4H_2O$. The pH of medium 271 was adjusted to 3.0 using H_2SO_4 1 M. Both cultures used CO_2 gas in the air as the carbon source for their autotrophic cell synthesis. The elemental sulfur ($10\ g\ L^{-1}$) and Fe(II) ($4.5\ g\ L^{-1}$) as $FeSO_4 \cdot 7H_2O$ were additionally provided as energy sources for *A. thiooxidans* and *A. ferrooxidans*, respectively. Both bacterial cultures were incubated in a shaking incubator at 30 °C and 200 rpm.

Before bioleaching experiment, the acclimation of both bacterial cultures with As-containing mine waste was done with a subculture of both bacteria in 500 mL Erlenmeyer flasks with a working volume of 200 mL. For this, 1% of the mine waste was additionally suspended into both subcultures at the initial of cultivation. The pH of both subcultures was then monitored during the incubation. When the pH of both subcultures dropped below 2.0, the subcultures were used as the inocula for bioleaching experiment.

The number of bacterial cells in the inocula was defined by counting colony-forming unit (CFU) on agar plate according to the method described previously (Nguyen et al., 2015a).

2.3. Bioleaching experiment in flasks

The bioleaching in flasks was carried out to compare the performance of *A. thiooxidans*, *A. ferrooxidans*, and a mix of them for As and Mn removal from mine waste. The inocula (1%, vol/vol) were obtained from acclimation process containing 7.5×10^6 CFU mL^{-1} of *A. thiooxidans* and 5.6×10^4 CFU mL^{-1} of *A. ferrooxidans*. The experiment factors were listed in Table 1. Medium (200 mL) was the medium 271 which contained the same ingredients with cultivating medium. Solid content (5%, weight/volume) was selected according to previous studies (Chen and Lin, 2010; Liu et al., 2007). All experimental sets were inoculated with 0.5% (weight/volume) of elemental sulfur powder and $4.5\ g\ L^{-1}$ of Fe(II) to provide the energy source for bacterial growth.

2.4. Bioleaching experiment in electrochemical cells

The electrochemical effect on bioleaching of tungsten mine waste was investigated using an electrochemical system which contained a cathode and an anode fixed in a single chamber (See the supplementary material, Fig. S1). Both cathode and anode were graphite rods (15 cm in length and 0.5 cm in diameter). The surface area of both electrodes contacting with solution was 11.5 cm^2. Potential differences between anode and cathode were applied through a programmable direct current (DC) power supply (OPE-303QI, ODA Technologies, Incheon, Korea). Experimental set-ups were summarized in Table 2. The inoculum (1%. vol/vol) used for this experiment was the mixture of both bacteria with the ratio of 1:1 obtained from acclimation process. The inoculum contained 7.5×10^6 CFU mL^{-1} of *A. thiooxidans* and 5.6×10^4 CFU mL^{-1} of *A. ferrooxidans*. The working volume, solid content, and elemental sulfur concentration were similar to the flask

Table 1
Experimental set-ups for flask bioleaching.

Exp. Set-up	Inoculum	Mine waste	Medium	Sulfur	Fe(II), g/L
Abiotic control	None	10 g (5%)	200 mL	1 g (0.5%)	4.5
Bioleaching-T	*A. thiooxidans*				
Bioleaching-F	*A. ferrooxidans*				
Bioleaching-M	Mixed culture[a]				

[a] Mixed culture of *A. thiooxidans* and *A. ferrooxidans* with the ratio of 1:1.